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Potassium Salts Inhibit Growth of the Cyanobacteria *Microcystis* spp. in Pond Water and Defined Media: Implications for Control of Microcystin-Producing Aquatic Blooms

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Ten metals were assayed in 21 Indian ponds which comprised three groups: (i) eutrophic alkaline ponds containing <2.5 mM potassium and thick growths of *Microcystis aeruginosa* or *Microcystis flos-aquae* during most of the year, (ii) equally eutrophic alkaline ponds containing >2.8 mM potassium and no detectable *Microcystis* growth, and (iii) oligo- or mesotrophic ponds with various potassium and hydrogen ion concentrations and no persistent *Microcystis* blooms. The effects of potassium on *Microcystis* growth were examined in filter-sterilized pond water and in defined culture media. A 50% reduction in the 10-day yield of cultured *M. aeruginosa* was observed in DP medium and pond water supplemented with 1 and 3 mM KCl, respectively. In contrast, the addition of 2 to 30 mM NaCl did not suppress the growth of *M. aeruginosa* in either DP medium or pond water. Both 5 mM KCl and 20 mM $KHCO_3$ in J medium strongly inhibited the growth of *M. flos-aquae* C3-9, whereas 5 to 30 mM NaCl had no effect and 20 mM $NaHCO_3$ was stimulatory. For pond water cultured with a mixture of *M. aeruginosa* and the duckweed *Wolffia arrhiza*, *M. aeruginosa* dominated in unsupplemented water and *W. arrhiza* dominated in water supplemented with 4.8 mM KCl. Implications for the ecology and control of *Microcystis* blooms are discussed.

The cyanobacterium *Microcystis*, a prevalent inhabitant of eutrophic alkaline lakes worldwide (9), is a public health and environmental hazard for several reasons. In particular, many *Microcystis aeruginosa* cultures and natural blooms produce microcystins (9), potent hepatotoxins and potential carcinogens (42) that inhibit protein phosphatases 1 and 2A (5, 9, 36). Additional toxic compounds or metabolic inhibitors that have been reported for *Microcystis* include an elastase inhibitor called microviridin (31), trypsin and plasmin inhibitors (17, 18, 29), a hydrophobic material that inhibits Ca^{2+} uptake by fish tissue (8), *Daphnia*-toxic compound (19), and other toxins (4, 16). Water from *Microcystis*-infested lakes can be toxic to fish (3, 8), aquatic invertebrates (6, 19, 34, 38), domestic animals (14, 24), and humans (11, 13, 40). Therefore, any procedure that can limit the extent of *Microcystis* blooms is worth investigating.

We present evidence that potassium ions strongly inhibit the growth of *Microcystis* and thus offer a new possibility for the regulation of *Microcystis* blooms. The experiments involved both field and culture studies which examined (i) whether the potassium concentrations in ponds with dense *Microcystis* growths differ from those in similarly eutrophic ponds that lack detectable *Microcystis* but often contain the duckweed *Wolffia arrhiza*, (ii) whether the addition of KCl to filter-sterilized water from a *Microcystis*-dominated pond influences the growth of *Microcystis* or *W. arrhiza* in that water, and (iii) whether the addition of KCl or $KHCO_3$ to each of two defined media inhibits the growth of either *Microcystis flos-aquae* or *M. aeruginosa*. Controls included parallel studies with NaCl and $NaHCO_3$. Field measurements were conducted in India, because the warm water temperatures promoted conditions under which dense *Microcystis* growths could be repeatedly found in the same ponds at almost all times of the year.

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MATERIALS AND METHODS

J medium and *M. flos-aquae* (*M. aeruginosa* f. *flos-aquae*) C3-9 have been described previously (10, 33). DP medium, pH 8.2, contained the following per liter: $NaNO_3$ (170 mg), K_2HPO_4 (17.4 mg), tricine (120 mg), H_3BO_3 (2.5 mg), $MgSO_4 \cdot 7H_2O$ (50 mg), $CaCl_2 \cdot 2H_2O$ (27 mg), $FeCl_2 \cdot 6H_2O$ (5 mg), $MnCl_2 \cdot 4H_2O$ (1.4 mg), and $(NH_4)_4Mo_7O_{24} \cdot H_2O$ (1 mg). Experiments were performed in low-metal plastic or occasionally in glassware that had been soaked in 5% HNO_3 and extensively rinsed with deionized water.

Ponds 1 (DLW Road), 2 (Hydel), 3 (Assi), 5 (Laxmi), 6 (Lat Bhairon), 7 (CHS 1), 8 (CHS 2), 9 (Durga), 12 (Suraj), 19 (DLW), and 20 (Manduadih) were within the city limits of Varanasi (25°20′N, 83°0′E), Uttar Pradesh, India. Pond 11 (Sagar Lake) was in Sagar (23°50′N, 78°40′E), Madhya Pradesh, India. Ponds 10, 13, and 18 were in the villages of Chori Bazaar, Adityanagar, and Jamunipur, respectively, near Varanasi. Ponds 4 (Ghamaha), 14 (Mulla Talab), 15 (Kas), 16 (Chitini 1), 17 (Bhadohi 1), and 21 (Chitini 2) were in Bhadohi, near Varanasi. Tables 1 and 2 list sampling dates and numbers of samples. Cyanobacteria, algae, and floating plants were identified and counted microscopically. For metal analysis, each 80-ml sample was acidified by the addition of 800 μl of concentrated HNO_3, digested by the method of Martin (26), quantitatively resuspended in 16 ml of 1% HNO_3, and appropriately diluted (for Na, K, Mn, and Fe analyses only) with deionized water. All metals except sodium and potassium were analyzed in a Perkin-Elmer 2380 atomic absorption spectrophotometer. Sodium and potassium were assayed in a Systronics Mediflame model 127 flame photometer.

To study the growth of naturally occurring organisms in variously supplemented pond waters, *M. aeruginosa* from pond 5 and *W. arrhiza* from pond 1 were obtained by filtration of surface samples in which each was the only detectable phototroph. The corresponding water samples were filter sterilized by passage through 0.22-μm-pore-size filters (Millipore) and adjusted to pH 9.1, the pH of pond 5 (pond 1 had a pH of 8.9). Pond water aliquots were inoculated with each organism, individually and in combination with the other organism. Portions of each culture were supplemented with NaCl and KCl to the total concentrations indicated below, which included the amounts in each original water sample. Inocula were 300 *W. arrhiza* plants and 5×10^3 *M. aeruginosa* colonies per 5 ml, equivalent to 0.12 A_{675} unit of each organism in a tube containing 5 ml. Triplicate 5-ml aliquots were incubated at 35°C and 100 microeinsteins $\cdot m^{-2} \cdot s^{-1}$ of natural sunlight, with a diel cycle of 12 h of light and 12 h of darkness. At day 10 of growth, *M. aeruginosa* colonies were counted microscopically in a Neubauer hemacytometer. At the same time, all *W. arrhiza* plants in each tube were removed, spread onto a microscope slide, and counted under a dissecting microscope.

M. flos-aquae C3-9 was cultured in J medium, pH 8.2, either unsupplemented

TABLE 1. Metal concentrations in ponds

Pond no.	Concn[a] (μM)							
	Cadmium	Chromium	Copper	Iron	Lead	Manganese	Nickel	Zinc
1[b]	0.037 ± 0.008	0.90 ± 0.06	2.27 ± 0.03	581 ± 22	0.76 ± 0.03	91 ± 7.2	1.51 ± 0.11	7.50 ± 0.41
2[b]	0.138 ± 0.011	3.08 ± 0.12	0.63 ± 0.01	370 ± 13	0.20 ± 0.02	73 ± 5.8	3.89 ± 0.07	2.37 ± 0.18
3[b]	0.004 ± 0.001	2.45 ± 0.05	1.55 ± 0.04	53 ± 4.1	0.35 ± 0.01	111 ± 9.1	3.39 ± 0.09	4.46 ± 0.10
4[c]	0.024 ± 0.005	0.24 ± 0.03	0.57 ± 0.01	58 ± 3.3	0.23 ± 0.02	267 ± 5.9	0.42 ± 0.01	1.63 ± 0.14
5[b]	0.002 ± 0.000	0.51 ± 0.01	0.59 ± 0.03	23 ± 2.0	0.54 ± 0.02	4.4 ± 0.0	0.64 ± 0.02	3.87 ± 0.22
6[b]	0.000 ± 0.000	1.70 ± 0.05	1.46 ± 0.02	84 ± 5.1	0.55 ± 0.03	10 ± 2.0	2.74 ± 0.12	1.76 ± 0.04
7[b]	0.016 ± 0.001	0.42 ± 0.01	0.30 ± 0.01	28 ± 1.2	0.17 ± 0.01	2.4 ± 0.8	0.20 ± 0.06	0.00 ± 0.00
8[b]	0.000 ± 0.000	2.79 ± 0.02	1.20 ± 0.05	17 ± 1.4	0.00 ± 0.00	2.1 ± 0.5	3.17 ± 0.15	0.72 ± 0.09
9[b]	0.000 ± 0.000	1.65 ± 0.05	0.42 ± 0.01	31 ± 2.2	0.42 ± 0.01	3.4 ± 0.3	1.80 ± 0.04	1.38 ± 0.07
10[c]	0.016 ± 0.003	0.21 ± 0.01	0.41 ± 0.02	63 ± 4.1	0.19 ± 0.01	29 ± 1.5	0.31 ± 0.02	1.30 ± 0.05
11[d]	ND[e]	ND	0.41 ± 0.07	ND	ND	ND	0.19 ± 0.01	ND
12[b]	0.027 ± 0.004	0.29 ± 0.01	1.68 ± 0.05	42 ± 2.7	0.90 ± 0.04	11 ± 0.5	0.32 ± 0.01	2.18 ± 0.13
13[b]	0.011 ± 0.002	0.33 ± 0.01	0.32 ± 0.02	30 ± 2.4	0.03 ± 0.01	8.7 ± 0.6	0.40 ± 0.01	1.18 ± 0.09
14[c]	0.003 ± 0.001	0.10 ± 0.01	0.11 ± 0.02	74 ± 1.4	0.03 ± 0.01	5.6 ± 0.3	0.81 ± 0.02	0.00 ± 0.00
15[c]	0.010 ± 0.002	0.64 ± 0.00	0.37 ± 0.03	37 ± 1.3	0.16 ± 0.03	2.1 ± 0.1	0.19 ± 0.00	0.39 ± 0.04
16[c]	0.009 ± 0.002	0.02 ± 0.00	0.00 ± 0.00	0.7 ± 0.0	0.01 ± 0.00	1.4 ± 0.3	0.17 ± 0.00	0.00 ± 0.00
17[c]	0.100 ± 0.009	0.94 ± 0.01	0.61 ± 0.04	230 ± 12	0.27 ± 0.01	18 ± 1.2	0.28 ± 0.01	2.58 ± 0.34
18[c]	0.013 ± 0.002	1.95 ± 0.04	0.30 ± 0.03	108 ± 11	0.16 ± 0.00	11 ± 0.9	0.27 ± 0.01	0.52 ± 0.02
19[b]	0.028 ± 0.004	0.38 ± 0.02	0.08 ± 0.01	19 ± 1.0	0.23 ± 0.02	0.0 ± 0.0	0.26 ± 0.00	0.00 ± 0.00
20[c]	0.000 ± 0.000	2.60 ± 0.03	0.42 ± 0.02	30 ± 1.8	0.00 ± 0.00	5.5 ± 0.3	3.23 ± 0.13	0.00 ± 0.00
21[c]	0.010 ± 0.001	0.15 ± 0.01	0.15 ± 0.01	172 ± 12	0.13 ± 0.03	4.5 ± 0.2	0.17 ± 0.03	0.37 ± 0.02

[a] Four surface samples per pond: duplicates at 2 m from shore on upwind and downwind sides. Values are means ± standard deviations.
[b] Sampled on 23 October 1993.
[c] Sampled on 12 December 1993.
[d] Sampled on 15 March 1994.
[e] ND, not determined.

or supplemented with the concentrations of NaCl, $NaHCO_3$, KCl, or $KHCO_3$ listed in Fig. 3. Triplicate 5-ml cultures were grown at 30°C, with continuous illumination at 20 microeinsteins $\cdot m^{-2} \cdot s^{-1}$. The absorbance at 675 nm was measured at timed intervals.

RESULTS

Table 1 shows the concentrations of eight metals in samples from 21 ponds. Table 2 lists sodium and potassium concentrations in 13 alkaline eutrophic ponds.

The prevalent cyanobacteria, algae, and floating plants in each pond were identified (Table 2). The predominant phototrophs in eight ponds (ponds 5 to 11 and 13) were the cyanobacteria *M. aeruginosa* and *M. flos-aquae*, which formed substantial growths throughout most of the year. Ponds 1 to 4 exhibited year-round surface growths of the duckweed *W. arrhiza*, sometimes accompanied by a few plants of another duckweed, *Spirodela polyrhiza*, but no detectable *Microcystis*. Pond 12 was dominated by the cyanobacterium *Oscillitoria*. No growths of cyanobacteria or *Wolffia* were observed in samples from the remaining eight ponds (ponds 14 through 21), which were a heterogeneous group of oligotrophic, mesotrophic, or acidic waters with various sparse populations that were not studied in detail. Subsequent experiments were focused on alkaline eutrophic ponds 1 through 13, which contained substantial growths of either cyanobacteria or duckweeds.

The *Microcystis*- and *Wolffia*-dominated ponds were similarly eutrophic and alkaline. The mean pH of the *Wolffia* ponds was 9.5, with a range of 8.0 to 11.1. The mean pH of the *Microcystis* ponds was 9.4, with a range of 8.3 to 10.1. Although sodium, copper, nickel, chromium, lead, and zinc were observed in roughly equivalent amounts in both pond types, the maximum concentrations of potassium, cadmium, iron, and manganese were at least 3.5 times greater in *Wolffia*- than in *Microcystis*-containing ponds (Tables 1 and 2). The highest concentration of potassium or manganese in any *Microcystis*-containing sample was less than the lowest concentration of that metal in any *Wolffia*-containing sample. In contrast, the concentration ranges of cadmium and iron overlapped for the two pond types.

To determine whether potassium, cadmium, iron, or manganese ions could influence the ability of *Microcystis* to grow in pond water, various concentrations of each metal chloride were added to aliquots of filter-sterilized water from *Microcystis*-containing pond 5. The tubes were inoculated with *Microcystis* from that pond. Of the four salts, only KCl strikingly inhibited *Microcystis* growth within the concentration ranges found in the ponds (Fig. 1). A 50 or 90% decrease in growth yield at 10 days was observed at a final concentration of 3 or 7 mM potassium, respectively, in comparison to the growth yield of unsupplemented controls containing 1 mM potassium (Fig. 1). The addition of equivalent amounts of NaCl did not affect growth. Interestingly, the 50% inhibitory potassium concentration (3 mM) was similar to the highest potassium concentration (2.4 mM) that was detected in any *Microcystis*-containing pond (Table 2).

To test whether added KCl could alter the relative prevalence of *Microcystis* and *Wolffia* in pond water cultures, filter-sterilized water samples from *Microcystis*-dominated pond 5 and *Wolffia*-dominated pond 1 were adjusted to pH 9.1 and inoculated with a mixture of *M. aeruginosa* from pond 5 and *W. arrhiza* from pond 1. The inocula (300 *W. arrhiza* plants and 5×10^3 *M. aeruginosa* colonies) corresponded to equivalent A_{675} units of each organism. One portion of water from pond 5 was supplemented with KCl to achieve the potassium concentration (4.8 mM) of the water from pond 1. After 10 days of incubation in triplicate tubes, each organism was microscopically counted. *W. arrhiza* predominated in water from pond 1 and in potassium-supplemented water from pond 5, whereas *M. aeruginosa* predominated in unsupplemented water from pond 5 (Fig. 2). Thus, the simple addition of potassium chlo-

TABLE 2. Sodium and potassium concentrations in alkaline eutrophic ponds[a]

Dominant organism (pH[b])	Pond no.	Date (day/mo/yr)	No. of samples[c]	Concn (mM)[d]		Na/K molar ratio	Dominant phototroph[e]
				Sodium	Potassium		
Wolffia (range, 8.0–11.1; mean, 9.5)	1	23/10/93	4	11.9 ± 0.3	5.3 ± 0.1	2.3	Dense *W. arrhiza*[f]
	1	26/06/94	4	8.1 ± 0.3	4.9 ± 0.1	1.6	Dense *W. arrhiza*[f]
	2	23/10/93	4	9.9 ± 0.3	5.1 ± 0.2	1.9	Dense *W. arrhiza*[f]
	3	23/10/93	4	7.9 ± 0.2	4.7 ± 0.8	1.7	Dense *W. arrhiza*[f]
	4	01/12/93	4	16.9 ± 0.9	8.6 ± 0.6	1.9	Dense *W. arrhiza*[f]
	Mean		20	10.9 ± 3.4	5.7 ± 1.4	1.9	Dense *Wolffia*[f]
Microcystis (range, 8.3–10.1; mean, 9.4)	5	16/10/93	4	11.2 ± 0.6	1.0 ± 0.1	11.0	Dense *M. aeruginosa*[g]
	5	23/10/93	4	10.2 ± 0.3	0.9 ± 0.1	11.3	Dense *M. aeruginosa*[g]
	5	31/03/94	8	9.7 ± 0.4	1.7 ± 0.2	5.6	Dense *M. aeruginosa*[g]
	5	16/05/94	8	12.0 ± 1.0	2.0 ± 0.2	6.2	Dense *M. aeruginosa*[g]
	5	26/06/94	4	11.3 ± 0.4	1.4 ± 0.1	8.0	Dense *M. aeruginosa*[g]
	6	23/10/93	4	8.8 ± 0.4	0.9 ± 0.1	9.6	Dense *M. aeruginosa*[g]
	6	31/03/94	8	14.3 ± 0.4	1.5 ± 0.4	9.4	Dense *M. aeruginosa*[g]
	7	23/10/93	4	11.9 ± 0.4	1.3 ± 0.2	9.3	Dense *M. flos-aquae*[g]
	7	31/03/94	8	13.3 ± 1.0	1.4 ± 0.1	9.4	Dense *M. flos-aquae*[g]
	8	23/10/93	4	12.1 ± 0.5	1.3 ± 0.2	9.2	Dense *M. aeruginosa*[g]
	9	23/10/93	4	6.6 ± 0.4	0.4 ± 0.1	15.7	Dense *M. aeruginosa*[g]
	Mean		60	11.4 ± 2.1	1.4 ± 0.4	9.5	Dense *Microcystis*[g]
	9	31/03/94	8	5.8 ± 0.3	0.7 ± 0.2	8.8	Some *M. aeruginosa*[h]
	10	01/12/93	4	17.8 ± 0.4	2.4 ± 0.1	7.4	Some *M. aeruginosa*[h]
	11	15/03/94	4	2.7 ± 0.3	0.6 ± 0.1	4.8	Some *M. aeruginosa*[h]
	13	23/10/93	4	5.7 ± 0.2	1.0 ± 0.2	5.7	Some *M. aeruginosa*[h]
	Mean		20	7.7 ± 4.3	1.1 ± 0.5	7.0	Some *Microcystis*[h]
Other cyanobacteria (8.8)	12	23/10/93	4	8.6 ± 0.4	3.1 ± 0.1	2.8	*Oscillatoria* sp.[i]

[a] All ponds whose pH was greater than 8 and that contained cyanobacteria or *W. arrhiza* are shown.
[b] pH varied between 8 and 11 in response to factors (such as time of day or amount of rainfall) that produced more variation than did the pond identity.
[c] 4, duplicate surface samples taken 2 m from the upwind and downwind sides of each pond; 8, duplicate surface samples taken 2 m from the four sides of each pond.
[d] Mean ± standard deviation.
[e] On the indicated sampling date; observed and counted microscopically.
[f] Dense *W. arrhiza*, most of the pond surface covered with *W. arrhiza* sometimes accompanied by a few plants of another duckweed, *S. polyrhiza*; no detectable *Microcystis*.
[g] Dense *Microcystis*, more than 10^4 *Microcystis* colonies per ml and approximately 10^3 cells per colony. No other prevalent cyanobacteria were detected in ponds 5, 7, 8, or 9. Pond 6 contained a minority population of 10^3 tricomes of *Spirulina* per ml of samples collected on 23 October 1993 but not on 31 March 1994. Most *Microcystis* colonies contained epiphytic *Navicula*.
[h] Some *Microcystis*, fewer than 5×10^3 *Microcystis* colonies per ml. Much of the surface of pond 13 was covered with the duckweed *Lemna*.
[i] 10^4 *Oscillatoria* tricomes per ml.

ride to pond water was sufficient to shift the dominance pattern from *M. aeruginosa* to *W. arrhiza*.

The effects of KCl, $KHCO_3$, NaCl, and $NaHCO_3$ on the growth of the pure culture of *M. flos-aquae* C3-9 (Fig. 3), as well as on the growth of *M. aeruginosa* from pond 5 (Fig. 1), were examined in two defined culture media, J and DP. A 5 mM concentration of KCl strongly inhibited the growth of both organisms (Fig. 1 and 3B), whereas 5 to 30 mM NaCl had no effect on either (Fig. 3B; data for *M. aeruginosa* not shown).

The potassium inhibition was not limited to KCl, since 20 mM $KHCO_3$ caused the death of the *M. flos-aquae* C3-9 culture (Fig. 3A). In contrast, 20 mM $NaHCO_3$ acted as a bicarbonate source that stimulated growth. $KHCO_3$ concentrations of less than 20 mM were not tested. Cultures with 20 mM $KHCO_3$ turned white within 2 days, a bleaching phenomenon which was more rapid than that observed with 5 mM KCl and roughly equivalent to that with 20 mM KCl.

DISCUSSION

The culture data indicate that potassium ions, added as either chlorides or bicarbonates, inhibit the growth of *M. flos-aquae* C3-9 and *M. aeruginosa* in both pond water and defined media, whereas comparable sodium salts at the same concentrations do not (Fig. 1 and 3). The 50 and 90% inhibitory concentrations in J medium, DP medium, and pond water were all between 1 and 7 mM. The 50% inhibitory concentration in pond water cultures (3 mM) was similar to the maximum potassium concentration (2.5 mM) observed in all 80 samples from eight *Microcystis*-containing ponds (Table 2). Twenty-four other samples from five ponds (ponds 1 to 4 and 12) that were also alkaline and eutrophic contained more than 2.8 mM potassium, but none had detectable *Microcystis* growth. It is therefore possible that the potassium concentration in the ponds studied influenced the prevalence of *Microcystis*. This idea is supported by experiments in which potassium chloride

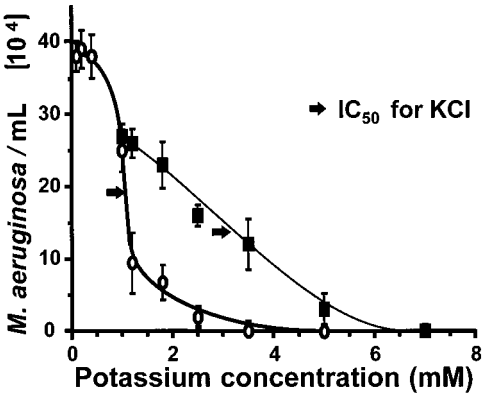

FIG. 1. Effect of potassium chloride concentration of the growth of field-collected *M. aeruginosa* in DP medium (○) or filtered water from pond 5 (■). Bars indicate standard deviations of triplicate cultures at 10 days of growth. IC_{50}, 50% inhibitory concentration. The ordinate shows the number of microscopically counted *M. aeruginosa* colonies per milliliter.

was added to filter-sterilized water from an *M. aeruginosa*-containing pond. Aliquots of KCl-supplemented and unsupplemented pond water were then inoculated with either *M. aeruginosa* alone or a mixture of *M. aeruginosa* and *W. arrhiza*. In both cases the addition of KCl, but not NaCl, inhibited the growth of *M. aeruginosa*. In the second case, *W. arrhiza* predominated in KCl-supplemented aliquots but not in unsupplemented or NaCl-supplemented aliquots. These observations indicate that the addition of KCl is sufficient to alter the prevalence of *M. aeruginosa* in pond water. The high-potassium and low-potassium ponds probably differed in other properties as well, but the potassium effect was striking (Fig. 1 and 2).

Although potassium inhibition of cyanobacterial growth has not been previously suggested for field situations, the effect on laboratory cultures confirms prior reports. In particular, Zehnder and Gorham (43) achieved the greatest yield of *M. aeruginosa* NRC-1 in modified Fitzgerald medium that contained one-half or one-fourth the usual KH_2PO_4 concentration and that exhibited Na/K ratios of 9 to 15. Those authors also observed that strain NRC-1 was inhibited if KNO_3 replaced

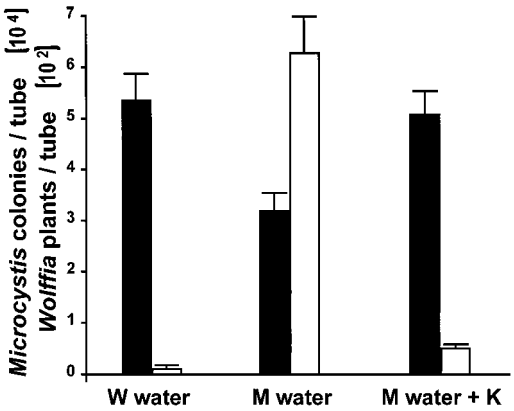

FIG. 2. Growth of field-collected *W. arrhiza* (black bars) and *M. aeruginosa* (white bars) in filtered pond water. W water, water from *Wolffia*-containing pond 1; M water, water from *Microcystis*-containing pond 5. Water from pond 5 was also supplemented with potassium chloride (M water + K) to a final concentration of 4.8 mM potassium, the potassium concentration in water from pond 1. *Microcystis* was microscopically counted in a hemacytometer. All *Wolffia* plants were removed from each tube and counted. Error bars represent standard deviations for triplicate cultures at 10 days of growth.

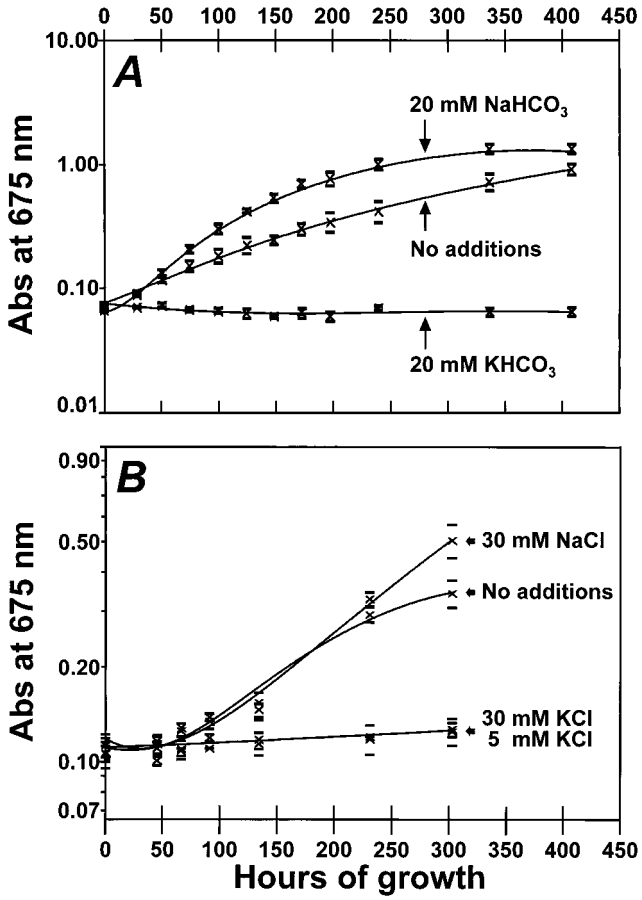

FIG. 3. Effects of $NaHCO_3$, $KHCO_3$, NaCl, and KCl on the growth of *M. flos-aquae* C3-9 in J medium. Bars represent standard deviations of data for triplicate cultures. Abs, absorbance.

two-thirds, but not one-third, of the 4.38 mM $NaNO_3$ in their medium no. 10, which also contained 0.8 mM sodium and 0.34 mM potassium from other compounds. The toxic replacement therefore yielded 2.25 mM sodium, 3.27 mM potassium, and an Na/K molar ratio of 0.69; the nontoxic replacement corresponded to 2.73 mM sodium, 1.79 mM potassium, and an Na/K ratio of 2.1. In contrast, the unmodified medium, which had been optimized for the growth of *Microcystis* (43), contained 5.18 mM Na and 0.34 mM K, for an Na/K ratio of 15.2. J medium, which we have adapted for the growth of *Microcystis* (10, 33), contains 9 mM Na and 0.44 mM K, yielding an Na/K ratio of 20. For comparison, no *Microcystis*-containing pond in these studies contained more than 2.4 mM potassium (Table 2). The mean Na/K ratio in ponds with dense *Microcystis* growths was 9.5 (Table 2).

Allen (1) states that four other cyanobacteria (i.e., *Synechococcus cedrorum*, *Chrococcus turgidus*, a *Chroococcus* sp., and an *Oscillitoria* sp.) grow in Allen 3 medium without added potassium and, furthermore, are inhibited by potassium in the absence of sodium. However, not all cyanobacteria exhibit potassium sensitivity, at least in certain media, since several genera can grow in Allen and Arnon medium (2, 43) with 20 mM KNO_3 and an Na/K ratio of 0.15. In contrast, neither *M. aeruginosa* NRC-1 (43) nor *M. flos-aquae* C3-9 (32a) survives in Allen and Arnon medium.

Potassium toxicity at the concentrations used here is surprising, since K^+ is the major cation inside cells. The cause of the

potassium sensitivity of *Microcystis* is unknown, but several plausible explanations involve potassium inhibition of sodium-related phenomena. Sodium is required for various aspects of cyanobacterial metabolism, including the uptake of bicarbonate (12, 20, 25, 27), the protection of photosystem II (44), and the maintenance of the intracellular pH (7, 15, 22, 23, 28, 32). The latter requirement is pronounced in alkalophiles, such as *Microcystis*, whereas the former two are possessed by many cyanobacteria. The sodium requirement of another alkalophilic cyanobacterium, *Spirulina*, increases with the increasing pH of the growth medium (37). For *Spirulina*, the largest lethal effect of sodium deprivation was recently reported to be an increase in the intracellular pH, although photosynthesis and bicarbonate incorporation were also influenced to some degree (37). Potassium ions are directly involved in the maintenance of internal pH in several other organisms (21, 22, 30, 35, 39, 41), but these are not alkalophiles.

It is unclear whether the potassium inhibition of *Microcystis* growth in Indian pond water depends on the absolute potassium concentration or on the Na/K ratio, since the sodium concentrations in most of the ponds studied were similar (Table 2). These sodium concentrations were too high to allow adequate experimental variation of the Na/K ratio in culture studies with pond water.

If potassium does indeed influence the occurrence of *Microcystis*, pollution management strategies that maximize potassium output and minimize sodium output might decrease the risk of microcystin-producing growths in affected waters. For example, most *Microcystis*-containing ponds in this study were contaminated with detergents and soaps used for laundry and bathing. Potassium-based instead of sodium-based detergents and soaps would be less likely to promote *Microcystis* blooms. The similar manipulation of fertilizers, barnyard runoff, and other effluents might decrease certain deleterious effects of nonpoint pollution.

Because the potassium concentrations that are toxic to *Microcystis* are not harmful to most other organisms, we propose that potassium compounds may be a safer alternative to copper sulfate or to other biocides that are currently added to water supplies to limit *Microcystis* blooms. The amounts of potassium that would have to be added might be excessive for large lakes with rapidly flowing water, but many of the ponds described here are small artificial enclosures (100 by 100 m or smaller) with inadequate outlets. The manipulation of potassium concentrations might affect the amount of *Microcystis* in these waters, which are extensively used by humans and domestic animals.

Potassium supplementation did not decrease the growth of the duckweeds in pond water (Fig. 2) and probably would not affect most true algae. Although duckweeds and algae can be nuisances, they do not produce microcystins (9, 36) or other toxic products of *Microcystis* (4, 8, 16–18, 19, 29, 31). Furthermore, the ingestion of *Microcystis* cells can be harmful to zooplankton (6, 19, 34, 38), which otherwise limit algal blooms.

An accurate assessment of the environmental role of potassium and of its potential for the control of microcystin-producing blooms awaits more detailed and comprehensive field studies than those presented here, which were limited to metals, surface water, and the predominant phototrophs in each sample. At present, the strongest evidence for a potassium effect is the inhibited growth of *Microcystis* in potassium-supplemented pond waters, as well as the general agreement between the laboratory and field results. The existing data suggest that the potassium ion concentration, which has been largely ignored in the past, is an important factor that deserves further investigation.

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